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(FILE 'HOME' ENTERED AT 09:29:43 ON 17 SEP 2002)

FILE 'REGISTRY' ENTERED AT 09:29:50 ON 17 SEP 2002

E N-TERT-BUTYLOXYCARBONYL-3,5-DIIODO-L-THYRONINE/CN

E N-ACTYL-3-iodo-L-TYROSINE/CN

E N-ACETYLPHENYLALANYL-3,5-DIIODO-L-TYROSINE/CN

L1 1 S E2

FILE 'CAPLUS' ENTERED AT 09:35:24 ON 17 SEP 2002

L2 3 S L1

L3 0 S L1 AND TYROSINE?

L4 17408 S ALANINE? AND TYROSINE?

L5 14 S L4 AND N-ACETYLPHENYL?

L6 7 S N-ACETYL-3-iodo-L-TYROSINE?

L7 7 S N-ACETYL-3-iodo-L-TYROSINE?

L8 7 DUP REM L7 (0 DUPLICATES REMOVED)

L9 7 S L8

L10 2 S L8 AND LABEL?

L11 0 S N-TERT-BUTYLOXYCARBONYL-3,5-DIIODO-L-THYRONINE?

L12 0 S N-TERT-BUTYLOXYCARBONYL-3,5-DIIODO-L-THYRONINE?

L13 0 S N-ACETYL-3,4-DIBROMO-L-TYROSINE?

L14 0 S N-ACETYLPHENYLALANYL-3,5-DIIODO-L-TYROSINE?

L15 12 S N-ACETYL-3,5-DIIODO-L-TYROSINE?

L16 12 DUP REM L15 (0 DUPLICATES REMOVED)

L17 12 S L16

L18 1 S L16 AND LABEL?

=>

Gab I, Gailene

T : STIC-ILL
Subj ct: 09/548,883

Please provide a copy of the following literature:

- 1) Block, Paul, Jr., Synthesis of 3-iodo-L-thyronine and its iodinated derivatives, J. Med. Chem. (1976), 19(8), 1067-9.
- 2) Michel, Raymond et al., Synthesis of 3,5-diiodo-L-thyronine labeled with tritium at 2' and 6' in hormone derivatives, Bull. soc. chim. biol. (1960), 42, 1207-11.
- 3) Frieden, Earl et al., The thyroxine-like activity of compounds structurally related to thyroxine, J. Biol. Chem. (1948), 176, 155-63.

Thanks a bunch,
Gail R. Gabel
7B15
305-0807

L8 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1976:457355 CAPLUS

DOCUMENT NUMBER: 85:57355

TITLE: Synthesis of 3-iodo-L-thyronine and its iodinated derivatives

AUTHOR(S): Block, Paul, Jr.

CORPORATE SOURCE: Dep. Chem., Univ. Toledo, Toledo, Ohio, USA

SOURCE: J. Med. Chem. (1976), 19(8), 1067-9

CODEN: JMCMAR

DOCUMENT TYPE: Journal

LANGUAGE: English

AB 3-Iodo-L-thyronine (I) [10468-90-3] was prepd. from N-

acetyl-3-iodo-L-tyrosine

amide [59302-19-1] by coupling with an anisyliodonium salt followed by hydrolysis with HBr-HOAc. Further iodination of I gave

3,3'-diiodo-L-thyronine [4604-41-5] and 3,3',5'-triiodo-L-thyronine

[5817-39-0]. Chromatog. systems for sepn. and purifn. of these compds. are described.

L8 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1976:457355 CAPLUS

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L18 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1961:118304 CAPLUS

DOCUMENT NUMBER: 55:118304

ORIGINAL REFERENCE NO.: 55:22223d-f

TITLE: Synthesis of 3,5-diiodo-L-thyronine **labeled** with tritium at 2' and 6' in hormone derivatives

AUTHOR(S): Michel, Raymond; Truchot, Roger; Tron-Loisel, Henri; Poillot, Bernard

CORPORATE SOURCE: Ecole med. pharm., Dijon, Fr.

SOURCE: Bull. soc. chim. biol. (1960), 42, 1207-11

DOCUMENT TYPE: Journal

LANGUAGE: Unavailable

AB cf. CA 50, 10044b. 3,3',5-Triiodo-L-thyronine and L-thyroxine **labeled** with tritium in 2' or 6' position, were prepd. by iodination of 3,5-diiodo-L-thyronine tritiated in 2' or 6' position. The synthesis of the latter compd. involved 4 steps. 3-Iodoanisole was reduced catalytically by T into meta-**labeled** anisole. This product was converted to bis(p-anisyl)iodonium bromide, which was condensed with N-acetyl-3,5-diiodo-L-tyrosine Et ester to yield radioactive O-methyl-N-acetyl-3,5-diiodo-L-thyronine Et ester. By hydrolysis with HI, this substance was transformed into 3,5-diiodo-L-thyronine, **labeled** at 2' or 6' position; the maximum specific mol. activity could reach one g. atom of T. 10 references.

TI Synthesis of 3,5-diiodo-L-thyronine **labeled** with tritium at 2' and 6' in hormone derivatives

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L17 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1949:11555 CAPLUS

DOCUMENT NUMBER: 43:11555

ORIGINAL REFERENCE NO.: 43:2327b-d

TITLE: The thyroxine-like activity of compounds structurally related to thyroxine

AUTHOR(S): Frieden, Earl; Winzler, Richard J.

SOURCE: J. Biol. Chem. (1948), 176, 155-63

DOCUMENT TYPE: Journal

LANGUAGE: Unavailable

AB 3,5-Diiodo-L-tyrosine (I), 3,5-diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)benzoic acid (II), 3,5-diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)-DL-phenylglycine (III), N-acetyl-DL-thyroxine (IV), and **N-acetyl-3,5-diiodo-L-tyrosine** (V), were tested for thyroxine-like activity on metamorphosis of tadpoles (cf. C.A. 22, 2410.2) and prevention of increase in thyroid gland wts. of thiouracil-fed rats (cf. C.A. 37, 5125.6). In tadpoles, activity increased in the order V, I, IV, II, III; by the goiter-prevention method, activity increased in the order: V, I, III, II, IV. These findings indicate a lack of specificity of the side-chain requirements, the min. structural requirements for amphibian and mammalian activity being the .omicron.-dihalogenophenolic diphenyl ether configuration, a hydroxy group ortho or para to the ether oxygen and a side chain contg. a functional group.

nicarly

From: Gabel, Gailene
Sent: Tuesday, September 17, 2002 10:14 AM
To: STIC-ILL
Subject: 09/548,883

Please provide a copy of the following literature:

- 1) Block, Paul, Jr., Synthesis of 3-iodo-L-thyronine and its iodinated derivatives, J. Med. Chem. (1976), 19(8), 1067-9.
- 2) Michel, Raymond et al., Synthesis of 3,5-diiodo-L-thyronine labeled with tritium at 2' and 6' in hormone derivatives, Bull. soc. chim. biol. (1960), 42, 1207-11.
- 3) Frieden, Earl et al., The thyroxine-like activity of compounds structurally related to thyroxine, J. Biol. Chem. (1948), 176, 155-63.

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- 3) Frieden, Earl et al., The thyroxine-like activity of compounds structurally related to thyroxine, J. Biol. Chem. (1948), 176, 155-63.

Thanks a bunch,
Gail R. Gabel
7B15
305-0807

ILE 'HOME' ENTERED AT 13:46:15 ON 09 SEP 2002)

FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 13:46:38
ON 09 SEP 2002

L1 0 S THYROID (6P) N!TERT!BUTYLOXYCARBONYL?
L2 15 S THYROID (6P) ?L!TYROSINE? OR ?L!THYRONINE?
L3 15 DUP REM L2 (0 DUPLICATES REMOVED)
L4 24305 S TRIIODOTHYRONINE? (6P) THYROXINE?
L5 125 S TRIIODOTHYRONINE? (4A) ANALOG?
L6 591 S THYROXINE (4A) ANALOG?
L7 35 S L5 (P) L6
L8 35 S L7 AND L4
L9 26 DUP REM L8 (9 DUPLICATES REMOVED)
L10 26 S L9 AND (?BUTYLOXYCARBONYL? OR ?ACETYLPHENYLALANYL? OR ?DIBRO
L11 26 DUP REM L10 (0 DUPLICATES REMOVED)
L12 8 S L9 AND (?BUTYLOXYCARBONYL? OR ?ACETYLPHENYLALANYL? OR ?DIBRO
L13 6 S L6 AND (?BUTYLOXYCARBONYL? OR ?ACETYLPHENYLALANYL? OR ?DIBRO
L14 0 S L5 AND (?BUTYLOXYCARBONYL? OR ?ACETYLPHENYLALANYL? OR ?DIBRO
L15 4 S L13 AND L4
L16 4 DUP REM L15 (0 DUPLICATES REMOVED)
L17 0 S N!TERT!BUTYLOXYLCARBONYL!3!5!DIIODO!L!THYRONINE?
L18 0 S N!TERT!BUTYLOXYCARBONYL!3!5!DIIODO!L!THYRONINE?
L19 0 S N!TERT!BUTYLOXYCARBONYL!3!!3!5!TRIIODO!L!THYRONINE?
L20 0 S N!TERT!BUTYLOXYCARBONYL!3!5!DIIODO!L!TYROSINE?
L21 0 S N!ACETYL!3!IODO!L!TYROSINE?

=>

L10 ANSWER 7 OF 26 MEDLINE
 ACCESSION NUMBER: 60223290 MEDLINE
 DOCUMENT NUMBER: 60223290
 TITLE: The metabolic effects of the acetic and propionic acid
analogs of thyroxine and
triiodothyronine.
 AUTHOR: HILL S R Jr; BARKER S B; McNEIL J H; TINGLEY J O; HIBBETT L
 L
 SOURCE: J Clin Invest, (1960 Mar) 39 523-33.
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 FILE SEGMENT: OLDMEDLINE
 ENTRY MONTH: 196012
 ENTRY DATE: Entered STN: 19990716
 Last Updated on STN: 19990716
 TI The metabolic effects of the acetic and propionic acid **analogs**
 of **thyroxine** and **triiodothyronine.**
 ST thyroxin - related compounds; **triiodothyronine** - related
 compounds
 RN 51-48-9 (THYROXIN); **6893-02-3 (TRIIODOTHYRONINE)**

L10 ANSWER 9 OF 26 MEDLINE
 ACCESSION NUMBER: 60130522 MEDLINE
 DOCUMENT NUMBER: 60130522
 TITLE: The binding to serum protein of acetic and propionic acid
analogues of thyroxine and
triiodothyronine.
 AUTHOR: CHRISTENSEN L K
 SOURCE: Endocrinology, (1960 Sept) 67 407-12.
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 FILE SEGMENT: OLDMEDLINE
 ENTRY MONTH: 196012
 ENTRY DATE: Entered STN: 19990716
 Last Updated on STN: 19990716
 TI The binding to serum protein of acetic and propionic acid
analogues of thyroxine and triiodothyronine.
 ST blood proteins - metabolism; thyroxin - related compounds;
triiodothyronine - related compounds
 RN 51-48-9 (THYROXIN); **6893-02-3 (TRIIODOTHYRONINE)**

L10 ANSWER 10 OF 26 MEDLINE
 ACCESSION NUMBER: 58053786 MEDLINE
 DOCUMENT NUMBER: 58053786
 TITLE: Studies of **thyroxine** and some of its
analogues. V. Metabolic activity in vitro and in
 vivo of the acetic acid **analogues** of
triiodothyronine and thyroxine.
 AUTHOR: WISWELL J G; ASPER S P Jr
 SOURCE: Bull. Johns Hopkins Hosp, (1958 Mar) 102 (3) 115-26.
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 FILE SEGMENT: OLDMEDLINE
 OTHER SOURCE: CLML5834-3299-597-605
 ENTRY MONTH: 195812
 ENTRY DATE: Entered STN: 20000825
 Last Updated on STN: 20000825
 TI Studies of **thyroxine** and some of its **analogues.** V.
 Metabolic activity in vitro and in vivo of the acetic acid
analogues of triiodothyronine and thyroxine.
 ST thyroxin - related compounds; **triiodothyronine** - related
 compounds
 RN 51-48-9 (THYROXIN); **6893-02-3 (TRIIODOTHYRONINE)**

L10 ANSWER 13 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1986:195746 BIOSIS

DOCUMENT NUMBER: BR30:107618

TITLE: **ANALOG FREE THYROXINE AND FREE TRIIODOTHYRONINE ASSAYS FROM DIAGNOSTIC PRODUCTS CORPORATION WITH IMPROVED PERFORMANCE IN PREGNANCY AND IN NONTHYROIDAL ILLNESS.**

AUTHOR(S): WITHERSPOON L R; SHULER S E; NEELY H R; GILBERT S

CORPORATE SOURCE: OCHSNER MED. INST., NEW ORLEANS, LA.

SOURCE: 31ST ANNUAL MEETING OF THE SOCIETY OF NUCLEAR MEDICINE (SOUTHWESTERN CHAPTER), DALLAS, TEX., USA, MAR. 13-16, 1986. J NUCL MED, (1986) 27 (2), 313.
CODEN: JNMEAQ. ISSN: 0022-3123.

DOCUMENT TYPE: Conference

FILE SEGMENT: BR; OLD

LANGUAGE: English

TI **ANALOG FREE THYROXINE AND FREE**

TRIIODOTHYRONINE ASSAYS FROM DIAGNOSTIC PRODUCTS CORPORATION WITH IMPROVED PERFORMANCE IN PREGNANCY AND IN NONTHYROIDAL ILLNESS.

RN 6893-02-3 (**TRIIODOTHYRONINE**)

51-48-9Q, 7488-70-2Q (**THYROXINE**)

L10 ANSWER 14 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1979:221714 BIOSIS

DOCUMENT NUMBER: BA68:24218

TITLE: A MODEL FOR THYROID HORMONE RECEPTOR INTERACTIONS.

AUTHOR(S): ANDREA T A; DIETRICH S W; MURRAY W J; ROTHENBERG S; KOLLMAN P A; JORGENSEN E C

CORPORATE SOURCE: DEP. PHARM. CHEM., SCH. PHARM., UNIV. CALIF., SAN FRANCISCO, CALIF. 94143, USA.

SOURCE: J MED CHEM, (1979) 22 (3), 221-232.

CODEN: JMCMAR. ISSN: 0022-2623.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB Theoretical electronic structure calculations on the thyroid hormones [**thyroxine, triiodothyronine**] and **analog**, as well as model hormone-receptor interactions, were carried out. The 4'-OH group is evidently a H-bond donor to the in vivo nuclear receptor and at the receptor this OH group is trans to the 3' (distal) substituent; there is an important intramolecular interaction between 3' and 4' substituents and those 3' substituents that most favor both 4' OH orientation trans to the 3' group and a more acidic OH group substantially increase binding and biological activity; there is a direct correlation between the conformational free energy of the aromatic rings and biological activity.

AB Theoretical electronic structure calculations on the thyroid hormones [**thyroxine, triiodothyronine**] and **analog**, as well as model hormone-receptor interactions, were carried out. The 4'-OH group is evidently a H-bond donor to the in. . .

L10 ANSWER 15 OF 26 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1978:195464 BIOSIS

DOCUMENT NUMBER: BA66:7961

TITLE: ANALYSIS OF THYROID HORMONES AS THEIR HEPTA FLUORO BUTYRYL METHYL ESTER DERIVATIVES BY GAS CHROMATOGRAPHY MASS SPECTROMETRY.

AUTHOR(S): PETERSEN B A; VOUIROS P

CORPORATE SOURCE: INST. CHEM. ANAL., APPL. FORSENIC SCI., NORTHWEST. UNIV., BOSTON, MASS. 02115, USA.

SOURCE: ANAL CHEM, (1977) 49 (9), 1304-1311.

CODEN: ANCHAM. ISSN: 0003-2700.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB The mass spectra of the heptafluorobutyryl methyl ester derivatives of

several thyroid hormones [**diiodothyronine**, **triiodothyronine** and **thyroxine**] and their **analogues** are examined and their fragmentation patterns are discussed. Ions of significant relative intensity dominate the high mass region of the spectra of these derivatives. In view of the decreased interference of background peaks at that high mass region, this feature is effectively utilized in the gas chromatography-mass spectrometry analysis of these compounds by selective ion monitoring. Detection limits of 0.5 pg in human serum can be attained.

AB The mass spectra of the heptafluorobutyryl methyl ester derivatives of several thyroid hormones [**diiodothyronine**, **triiodothyronine** and **thyroxine**] and their **analogues** are examined and their fragmentation patterns are discussed. Ions of significant relative intensity dominate the high mass region of the. . .

=>

L9 ANSWER 10 OF 26 USPATFULL

ACCESSION NUMBER: 94:3704 USPATFULL
TITLE: Method for measuring the free fraction of ligands in biological fluids
INVENTOR(S): Midgley, John E., Great Missenden, England
Sheehan, Christopher P., Cardiff, England
Christofides, Nicos D., Cardiff, England
PATENT ASSIGNEE(S): Amersham International PLC, Buckinghamshire, Great Britain (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5278080		19940111
APPLICATION INFO.:	US 1993-22416		19930211 (8)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1992-885070, filed on 18 May 1992, now abandoned which is a continuation of Ser. No. US 1990-551580, filed on 2 Jul 1990, now abandoned which is a continuation-in-part of Ser. No. US 1990-473964, filed on 17 Apr 1990, now abandoned		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Nucker, Christine M.		
ASSISTANT EXAMINER:	Stucker, Jeffrey		
LEGAL REPRESENTATIVE:	Wenderoth, Lind & Ponack		
NUMBER OF CLAIMS:	9		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	3 Drawing Figure(s); 3 Drawing Page(s)		
LINE COUNT:	1303		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A one-step assay for the free portion of a ligand in a biological sample involves incubating a mixture of the sample with a labelled antibody for the ligand and a ligand analogue which competes with the ligand for binding to the antibody. The assay is characterized by choosing a ligand analogue which has a lower affinity for the antibody than does the ligand. An insolubilised ligand analogue preferably has a binding affinity for the antibody from 0.01% to 10% of that of the ligand. Ligand/ligand analogue pairs exemplified are T4/T3 (Thyroxine/Triiodothyronine); Testosterone/etiocholanol; and T3/T2.

SUMM . . . role of thyroid hormones and their associated binding proteins in determining thyroid activity and clinical status in thyroid disease. For **thyroxine**, about 99.98% of the total hormone in the circulation is in a protein-bound state, and for the accompanying hormone **triiodothyronine**, about 99.7% is similarly protein-bound. Three naturally occurring proteins in blood serum or plasma will bind **thyroxine** and **triiodothyronine**, accounting for virtually all the protein-bound hormones: these are **thyroxine**-binding globulin (TBG), **thyroxine**-binding prealbumin (transthyretin, TBPA) and albumin (A). Nevertheless, it is now recognized that the severity of thyroid dysfunction is better correlated.

SUMM . . . serum or plasma and are in current use in many laboratories. Direct ligand assays, more especially those for serum free **thyroxine** and free **triiodothyronine**, are characterized by the measurement of the free ligand itself, rather than by other methods which are correlated to the free ligand concentration by a calculation, such as the free **thyroxine** index. Virtually all direct free ligand assays rely on the fact that the removal of a negligibly small portion of . . . that was present originally in the serum or plasma before the addition of the specific ligand binder. For the ligand **thyroxine** or **triiodothyronine**, less than 5% of the total available ligand (in protein-bound or free form) should be sequestered by the specific ligand.

SUMM In the method first developed commercially by Clinical Assays (GB

analogue) by activation of the cellulose using butane-1,4-diol diglycidyl ether. Differential binding ligand analogue coated polystyrene or cellulose particles not containing ferromagnetic cores can be separated in the assay system by centrifugation techniques. Alternatively, the triiodothyronine or other suitable **thyroxine**-related ligand **analogue** with desired properties can be covalently joined to proteins through amide bonds, using standard chemical techniques, preferably, but not necessarily, through the **triiodothyronine** or ligand **analogue** amino group. The protein-ligand analogue may be insolubilised by absorption on to the internal walls of plastic tubes, or be . . .

DETD CHEMISTRY OF SYNTHESIS OF **TRIIODOTHYRONINE**-DERIVATISED CELLULOSE PARTICLES (THE DIFFERENTIAL BINDING LIGAND ANALOGUE COMPLEX)

DETD The activated cellulose is now reacted with **triiodothyronine**, to give two possible stereoisomers: ##STR4##

DETD . . . centrifugation and resuspension into 20 mls 0.05M sodium carbonate/bicarbonate buffer, pH 9.6. Then, 13.3 milligrams of the sodium salt of L-**triiodothyronine** was added (using a solution in dimethylformamide). The mixture was incubated at 37.degree. C. for 3 hours. The particle suspension. . .

DETD SOURCE AND PROPERTIES OF **THYROXINE**-SPECIFIC MONOCLONAL ANTIBODY

DETD A preparation of **thyroxine**-specific monoclonal antibodies derived from a mouse hybridoma was obtained from Immunosearch Inc, Toms River, New Jersey, USA. This clone (no. . . . was achieved by column chromatography using diethyl-(aminoethyl)cellulose (DEAE), according to the suppliers protocols. The affinity constants of the antibody for **thyroxine**, **triiodothyronine**, and the **triiodothyronine**-complexed cellulose (see above) were measured by classical Scatchard analysis. The association constants at 37.degree. C. were a) for **thyroxine** 4.6.times.10.sup.9 L/mole, (quoted as approximately 0 L/mole by the supplier), b) for **triiodothyronine** 3.4.times.10.sup.7 L/mole (suggesting approximately 1% cross-reactivity for antibody binding compared with **thyroxine**), and c) for the **triiodothyronine**-cellulose complex used as solid phase in the free tyroxine assay 6.7.times.10.sup.5 L/mole. For comparison, a specimen of monoclonal anti-**thyroxine** antibody was taken through the standard iodination procedure for the production of iodinated-antibody, as described below, except that nonradioactive iodide. . . was substituted for the radioactive material. On purification, this iodinated antibody gave the following association constants at 37.degree.: a) for **thyroxine**, 4.4.times.10.sup.9 L/mole, b) for **triiodothyronine** 4.0.times.10.sup.7 L/mole and c) for the **triiodothyronine**-cellulose complex used as solid phase in the assay, 5.5.times.10.sup.5 L/mole. For the iodinated and noniodinated antibody preparations, the affinities of the antibody were virtually identical for the same substances, and for **thyroxine** the antibody affinity was in each case much higher than for its cross-reacting analogue **triiodothyronine**. Complexing of **triiodothyronine** to the cellulose particles reduced the affinity of the antibody for the complex still further, presumably due largely to additional. . . "bulky molecule" effects as described earlier. A further contribution to the apparently grossly lowered affinity of the antibody for the **triiodothyronine**-cellulose complex could be due additionally to the unavailability of a portion of the complexed **triiodothyronine** residues for binding by the antibody. However, the affinity of the antibody for **thyroxine** was considerably smaller than specified in W 83/03306 as being essential for a viable free **thyroxine** assay. The monoclonal antibody preparation was stored in the buffer as received from the supplier (see above) until required, at. . .

DETD PREPARATION OF 125-I LABELLED ANTI-**THYROXINE** ANTIBODIES

DETD . . . (12.9-22.2 GBq/ml) in 0.1M sodium phosphate buffer pH 6.0 containing 0.3M NaCl; 0.6 ml of a solution of the monoclonal anti-

thyroxine antibody preparation, concentrated to 5 mg/ml by freeze-drying and resuspension in 0.1 ml 0.1M sodium phosphate buffer, pH 7.5. Then, . . . an ultraviolet absorbance detector at 280 nm, and the matching radioactivity profile of the labelled antibody was followed. The [125-I]-labelled anti-**thyroxine** antibody preparation was collected in 2-3 ml fluid, eluting from the column after about 20 minutes. The specific activity of. . .

DETD DESCRIPTION OF THE IMMUNOMETRIC ASSAY FOR SERUM/PLASMA FREE

THYROXINE

DETD 50 microliters of a serum sample was mixed with 0.5 ml of the solid-phase **triiodothyronine** (T3)-conjugated cellulose complex suspension (concentration 0.5 g/L, see paragraph on preparation for the working strength concentration) containing 253 pmol complexed T3. 0.5ml [125-I]-labelled anti-**thyroxine** antibody solution (containing 3 ng [20 fmol] antibody) was then added. The molar ratio of complexed T3 to [125-I]-labelled antibody was approximately 12600/1. The solution was vortex mixed, and was incubated at 37.degree. C. for 30 minutes. Free **thyroxine** in the serum competed with the T3-cellulose complex for binding the [125-I]-labelled anti-**thyroxine** antibody, and the fraction of [125-I] counts bound to the complex was inversely proportional to the serum free **thyroxine** concentration. The magnetised T3-cellulose complex with associated [125-I]-antithyroxine antibody was now precipitated by placing the tubes containing the reaction mixture. . . . particles were then counted for 60 seconds in the usual way, using a standard radioisotope detector for [125-I] emissions. The free **thyroxine** concentrations of unknown sera were interpolated from a dose-response curve, constructed using samples with known free **thyroxine** concentrations and spanning the whole assay range of expected values. A typical dose-response curve is shown in FIG. 1.

DETD Assays using other preparations of anti-**thyroxine** monoclonal antibodies, with association constants for **thyroxine** of about 10×10^8 L/mole, were impractical, owing to a low B(0) of <5%, using T3-cellulose, indicating that less avid antibodies were. . . . range of antibody association constants for binding with the ligand analogue-cellulose solid phase complex, giving usable dose-response relationships for free **thyroxine** estimation, is thus 10^5 to 10^8 L/mole. This covers lower limits, below which stable binding of the antibody to the. . . . may be unattainable, and upper limits, above which the association constant of an antibody for the complex approaches that for **thyroxine**, thus limiting the desirable amount of ligand analogue-cellulose complex (see earlier argument).

DETD . . . antibody and the concentration (C) of the T3-cellulose solid phase complex was 5.5×10^5 times 10^5 times 2.53×10^{-10} or 1.39×10^5 times 10^{-4} . Similarly for the free **thyroxine**, the corresponding product was 4.6×10^9 times 1.5×10^{14} or 6.9×10^5 (if it is assumed that about 0.3% of the available hormone in 0.05 ml of a euthyroid serum containing 10×10^{-7} mole/L **thyroxine** is sequestered by the antibody). The close similarity of the $K_{ab} \times C$ product values for the competing T3-cellulose complex and sequestered **thyroxine** is a demonstration of the approximate equivalence of the effective avidity of the antibody for either moiety and predicts a. . . .

DETD . . . of approach to equilibrium of the labelled antibody bound to the T3-cellulose solid phase, using sera with various known free **thyroxine** concentrations. The assay had virtually achieved full equilibrium by 30 minutes at 37.degree. C.

DETD The affinity constant of the iodine-labelled antibody for **thyroxine** in the assay was $<5 \times 10^9$ L/mole. According to the teaching of Patent Application WO 83/03306, and additional writings by its. . . . the use of antithyroxine antibodies with affinity constants well below the value given by the reciprocal of the serum free **thyroxine** concentration (typically about 1.3×10^{11} mol/L) should give extremely insensitive and unusable dose-response

curves.

DETD Inspection of the equation describing the binding of **thyroxine** to the antibody in a free **thyroxine** assay (see earlier) reinforces this expectation, if it is assumed that the assay proceeds purely by classical Mass Action equilibrium principles. In the denominator of the equation $[FT4] \cdot K_{ab} \cdot P_{ab} / (1 + K_{ab}[FT4])$ as given earlier to describe the binding of free **thyroxine** to the antibody, a value for K_{ab} much less than $10 \cdot 10^{11}$ L/mole makes the magnitude of $K_{ab}[FT4]$ much less than unity. . . .

DETD . . . considering that, throughout the reaction of the labelled antibody with the competing solid phase T3-cellulose complex and the serum free **thyroxine** (continually released from the serum **thyroxine**-binding proteins as it is taken up by the antibody), the effective concentrations of both competing moieties do not essentially alter over the course of the binding reaction with the labelled antibody. Owing to the negligibly small fraction of **thyroxine** taken up by the labelled antibody, the free **thyroxine** concentration is virtually unaltered by readjustment of the serum free-bound equilibria, and the very large molar excess of the weak. . . the same over the course of the reaction. There is thus a simple competition between a constant concentration of free **thyroxine** and free T3-cellulose binding sites for binding the antibody, based only on their relative affinities and concentrations. Thus, the forward rates of association of the labelled antibody to either free **thyroxine** or unbound sites on the T3-cellulose binding complex are of the form $K(a) [fAn] [fPab]$, where $K(a)$ is either the association constant. . . .

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FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 11:40:39
ON 20 FEB 2002

L1 0 S ACETYL!3!iodo!L!TYROSINE
L2 0 S (?TYROSINE OT ?THYRONINE) (P) (TSH OR (THYROID STIMULATING HO
L3 11515 S (?TYROSINE OR ?THYRONINE) (P) (TSH OR (THYROID STIMULATING HO
L4 705 S (?TYROSINE) (P) (TSH OR (THYROID STIMULATING HORMONE))
L5 1541 S (?TYROSINE) (P) (THYROXINE OR TRIIODOTHYRONINE)
L6 136 S L4 AND L5
L7 0 S (?BUTYLOXYCARBONYL? OR ?ACETYLPHENYLALANYL? OR ?DIBROMO?) (P)
L8 63818 S (?BUTYLOXYCARBONYL? OR ?ACETYLPHENYLALANYL? OR ?DIBROMO?)
L9 97 S L8 AND L5
L10 96 DUP REM L9 (1 DUPLICATE REMOVED)
L11 37 S L10 AND (?ACETYL? AND ?iodo?)
L12 37 DUP REM L11 (0 DUPLICATES REMOVED)
L13 2 S L12 AND THYROID
L14 7 S L9 AND THYROID
L15 7 DUP REM L14 TEM (0 DUPLICATES REMOVED)
L16 0 S ?ACETYL!!!iodo!!!TYROSINE
L17 0 S ACETYL!!!iodo!!!TYROSINE
L18 6 S ACETYL (3A) IODO (3A) TYROSINE
L19 4 DUP REM L18 (2 DUPLICATES REMOVED)

L19 ANSWER 1 OF 4 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 1
AB During the catalytic dehalodeuteration of N-**acetyl**-3,5-di-
iodo-L-tyrosine amide solvent hydrogen was incorporated
instead of deuterium up to an order of 70%. The major part of the solvent.
. .

L19 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
IT Miscellaneous Descriptors
ANTI TRYPSIN P NITRO BENZYL ETHER N **ACETYL** DI **iodo**
TYROSINE POPULATION GROWTH

L19 ANSWER 3 OF 4 MEDLINE DUPLICATE 2
AB A small bifunctional antigen (4-hydroxy-5-**iodo**-3-nitrophenyl)
acetyl-epsilon-aminocaproyl-L-**tyrosine**
-azobenzene-p-arsonate [NIP-cap-TYR(ABA)] was found to induce fair humoral
antibody formation against NIP-cap but very little anti-ABA-TYR. This was
observed in rats. . .

L19 ANSWER 4 OF 4 MEDLINE
TI KINETICS OF IODINATION. II. GENERAL BASE CATALYSIS IN THE IODINATION OF
N-**ACETYL**-L-**TYROSINE** AND N-**ACETYL**-3-**iodo**-L-
TYROSINE.

2030290), the endogenous protein binders of **thyroxine** in serum or plasma are prevented from interfering in the estimation of the free fraction of **thyroxine** by first incubating the serum or plasma in a tube whose inner surface is coated with the immobilized specific ligand binder (an antibody raised against, and specific for, **thyroxine**). Conditions are arranged so that a very small amount of **thyroxine** (considerably less than 5%) is sequestered by the specific ligand binder immobilized on the tube walls. This ensures that the removal of **thyroxine** from the serum equilibrium system of free and protein-bound ligand is small enough not to significantly affect the original endogenous. . . off or aspiration, and a second incubation is performed in the tube with a prescribed amount of buffer containing radiolabelled **thyroxine**, when the binding sites of the specific ligand binder not already occupied by **thyroxine** from the first incubation are now occupied by the radiolabelled **thyroxine**. Since the fractional occupation of the binding sites of the specific ligand binder in the first incubation is proportional to the endogenous free ligand (**thyroxine**) concentration, the further occupation of otherwise vacant sites by radiolabelled **thyroxine** is inversely proportional to the original free ligand concentration. This method has the advantage that reagents well-known in the estimation of total **thyroxine** concentrations in serum or plasma can be used in this method of estimating the free ligand (**thyroxine**) concentration. It has however the disadvantage of requiring two sequential incubations to achieve the estimation, and may also be prone. . .

SUMM . . . independent of the concentration of such endogenous binders, the assay will show some degree of correlation. Present assays for free **thyroxine** and free **triiodothyronine** developed using this technique have been successful in respect of their independence of variations in endogenous concentrations of TBG and TBPA, but it has proved more difficult to achieve sufficiently reduced affinity of the analogue of **thyroxine** for albumin binding sites to avoid some weak correlation of free **thyroxine** assay values with serum albumin concentration. Additionally, the assay is affected by **thyroxine**-specific autoantibodies that occur rarely in high concentration and with high avidity in some sera, since these antibodies can sequester the **thyroxine** analogue strongly, and remove it from the assay. Finally, the method requires the synthesis of specifically designed tracer analogues suitable. . .

SUMM . . . addition, according to the patent applications and additional writings by its author, it is necessary (in an assay for free **thyroxine**) to use antibodies of a well-defined, suitably high avidity (association constant about 10×10^{11} L/mole) This requirement was conceived because the. . . the labelled antibody. Thus, the Mass Action term describing the distribution of the ligand bound to the antibody directed against **thyroxine** at equilibrium is in the form:

SUMM [FT4]=the free ligand (**thyroxine**) concentration,
SUMM K_{ab} =the association constant of the antibody for **thyroxine**,
and

DETD . . . be expected if the ligand analogue residue in the differential binding ligand analogue complex binds strongly. In the case of **thyroxine**, the use of the strongly binding ligand **thyroxine** in the differential binding ligand analogue complex could promote interference by the endogenous protein binding receptors, whereas its homologue **triiodothyronine**, being a weak binder in comparison, would most likely not do so.

DETD For an assay for the ligand free **thyroxine**, suitable materials to form an insoluble differential binding ligand analogue complex include polystyrene latex particles, (sometimes, but not essentially, containing. . . cores to enable separation of the differential binding ligand analogue complex by magnetic separation techniques) which is covalently linked to L-**triiodothyronine** (or other ligand

L14 ANSWER 1 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AB The mechanism of action of **thyroid** hormones on bone is still not clear. At low concentrations, they stimulate bone formation; at high concentrations, they elicit bone resorption in vitro and in vivo. In the present study we investigated the effect of T-3 and T-4 as well as their active and inactive analogs (TRIAC, SKF L-94901, rT-3, and DIT) on the IGF-I and TNF-alpha content in the medium of UMR-106 rat osteoblastic cells and fetal rat limb bones. In the dose-response studies, a biphasic increase in medium IGF-I was observed in both cells and limb bones, with peak stimulatory concentrations of 10^{-8} M for T-3 and 10^{-7} M for T-4 in both systems. At higher concentrations, at which **thyroid** hormones elicit bone resorption, the stimulatory effect diminished and finally was no longer detectable. The active analogs TRIAC and SKF L-94901 also enhanced IGF-I release in UMR-106 cells. The inactive compounds rT-3 and DIT failed to increase IGF-I in these cultures. The protein content of the cell culture wells exposed to high concentrations of **thyroid** hormones was similar to those containing low concentrations, indicating that the decrease in IGF-I content at high doses was not due to toxic effects. This was also confirmed by trypan blue exclusion. Time course studies with UMR-106 cells revealed a significant increase in medium IGF-I after 2 days of incubation. No significant further increase was observed after this up to 5 days of culture. In contrast, the medium of limb bone cultures showed a linear increase in IGF-I content up to 7 days of culture. No TNF-alpha production was observed in either UMR-106 cells or fetal limb bones. Also, no increase in medium TNF-alpha levels was seen in response to **thyroid** hormones. Based on our results, we conclude that IGF-I may be responsible for some of the anabolic effects of **thyroid** hormones in bone tissue, but TNF-alpha, at least in the models we used, does not play a role in the mediation of **thyroid** hormone action.

L14 ANSWER 2 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AB In vitro, the synthetic flavonoid EMD 21388 appears to be a potent inhibitor of **thyroxine** (T4) 5'-deiodinase and diminishes binding of T4 to transthyretin. In this study, in vivo effects and long-term administration of EMD 21388 on **thyroid** hormone production and metabolism were investigated. Intact male rats received EMD 21388 ($20 \mu\text{mol} \cdot \text{kg body wt}^{-1} \cdot \text{rat}^{-1} \cdot \text{day}^{-1}$) for 14 days. [^{125}I]T4 and 3,5,3'-[^{131}I]**triiodotyrosine** (T3) were infused continuously and intravenously in a double-isotope protocol for the last 10 and 7 days, respectively. EMD 21388 decreased plasma **thyroid** hormone concentrations, but thyrotropin levels in plasma and pituitary did not change. Plasma clearance rates for T4 and T3 increased. Thyroidal T4 secretion was diminished, but T3 secretion was elevated. Extrathyroidal T3 production by 5'-deiodination was lower. T4 concentrations were markedly lower in all tissues investigated. Total tissue T3 was lower in brown adipose tissue, brain, cerebellum, and pituitary, tissues that express the type II 5'-deiodinase isozyme due to decreased local T3 production. Most tissues showed increased tissue/plasma ratios for T4 and T3. These results indicate that this flavonoid diminished T4 and increased T3 secretion by the **thyroid**, probably in analogy with other natural flavonoids, by interference with one or several steps between iodide uptake, organification, and hormone synthesis.

L14 ANSWER 3 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AB The clinical experience from several years standing in the use of **bromotyrosine** for a variety of endocrine and inflammatory disorders of the **thyroid**. In view of its ability to modulate the physiological activity of the hypothalamo-pituitary-**thyroid** feedback and to improve the T1/T4 [**triiodothyronine**/**thyroxine**] ratio in peripheral tissues, the drug proved remarkably useful in the following disorders: normally functioning goiter with hyperthyroid tendency; hyperthyroid goiter; mononodular hyperplasia during

stabilization; and thyroiditis. **Dibromotyrosine** is a drug with adjuvant properties in morbid conditions of hyperthyroidism, particularly those of the Basedow type, during pregnancy, and during iodine prophylaxis of diffuse macro-micronodular goiter. Its easy handling and the absence of side effects make it an essential component of the medical management of the **thyroid** disorders.

L14 ANSWER 4 OF 7 USPATFULL

AB Disclosed are lantionine peptides having the structure ##STR1## methods of their preparation and use as pharmacologically active agents.

L14 ANSWER 5 OF 7 USPATFULL

AB Therapeutic compounds and methods for inhibiting amyloid deposition in a subject, whatever its clinical setting, are described. Amyloid deposition is inhibited by the administration to a subject of an effective amount of a therapeutic compound comprising an anionic group and a carrier molecule, or a pharmaceutically acceptable salt thereof, such that an interaction between an amyloidogenic protein and a basement membrane constituent is inhibited. Preferred anionic groups are sulfonates and sulfates. Preferred carrier molecules include carbohydrates, polymers, peptides, peptide derivatives, aliphatic groups, alicyclic groups, heterocyclic groups, aromatic groups and combinations thereof.

L14 ANSWER 6 OF 7 USPATFULL

AB The present invention relates to methods of delivering pharmaceutical agents across membranes, including the skin layer or mucosal membranes of a patient. A pharmaceutical agent is covalently bonded to a chemical modifier, via a physiologically cleavable bond, such that the membrane transport and delivery of the agent is enhanced.

L14 ANSWER 7 OF 7 USPATFULL

AB Prodrugs of bio-active hydroxyaromatic drugs having the structural formula:

A pharmaceutically acceptable prodrug of a biologically active, therapeutically effective hydroxyaromatic drug, said prodrug being selected from the group consisting of, (A) compounds having the structural formula:

DRUG--O--CR'R"--Z].sub.n

wherein:

DRUG --O-- is the hydroxyaromatic O-dehydro residue of said drug;

R' and R' may be the same or different and may be H, alkyl, aryl or electron withdrawing groups;

Z is a displaceable leaving group; and

n is an integer in the range of from 1 to 3, and (B) pharmaceutically acceptable salts thereof.

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L19 ANSWER 1 OF 4 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 1
 ACCESSION NUMBER: 89182963 EMBASE
 DOCUMENT NUMBER: 1989182963
 TITLE: Investigation on solvent hydrogen transfer during the catalytic deuteration of n-acetyl-3,5-di-iodo-l-tyrosineamide.
 AUTHOR: Oehlke J.; Niedrich H.; Zopfl H.-J.; Franke P.
 CORPORATE SOURCE: Institute of Drug Research, Academy of Sciences, Berlin-1136, Germany
 SOURCE: Journal of Labelled Compounds and Radiopharmaceuticals, (1989) 27/8 (861-868).
 ISSN: 0362-4803 CODEN: JLCRD4
 COUNTRY: United Kingdom
 DOCUMENT TYPE: Journal
 FILE SEGMENT: 023 Nuclear Medicine
 029 Clinical Biochemistry
 LANGUAGE: English
 SUMMARY LANGUAGE: English
 AB During the catalytic dehalodeuteration of N-acetyl-3,5-di-iodo-L-tyrosine amide solvent hydrogen was incorporated instead of deuterium up to an order of 70%. The major part of the solvent hydrogen was shown to be introduced into the reaction product independently of the dilution of the reacting gas. This direct transfer depends on type of solvent and catalyst and on the catalyst-to-substrate ratio in the same manner as found from deuterations of 4-halogenated phenylalanine derivatives and dehydroproline. The distribution of deuterium in the reaction products was determined by mass spectrometry.
 AB During the catalytic dehalodeuteration of N-acetyl-3,5-di-iodo-L-tyrosine amide solvent hydrogen was incorporated instead of deuterium up to an order of 70%. The major part of the solvent.

L19 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 ACCESSION NUMBER: 1978:196603 BIOSIS
 DOCUMENT NUMBER: BA66:9100
 TITLE: THE INFLUENCE OF ANTI METABOLITES ON TRICHOMONAS-VAGINALIS AS AN EXPERIMENTAL MODEL.
 AUTHOR(S): CHRISTOW C P
 CORPORATE SOURCE: GUTENBERGST. 30, 32 HILDESHEIM 1, W. GER.
 SOURCE: MICROBIOS, (1976 (RECD 1977)) 17 (68-69), 87-92.
 CODEN: MCBIA7. ISSN: 0026-2633.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB The relationship between antimetabolites and T. vaginalis was studied. In all the strains tested with p-nitrobenzylether of N-acetyldiiodotyrosine, growth was identical to that of the control series at concentrations up to 2 mg/ml. At higher concentrations a decrease in multiplication was noted. In the experimental series with antitrypsin, from 1 mg/ml produced an inhibitory effect on the Trichomonas population.
 IT Miscellaneous Descriptors
 ANTI TRYPSIN P NITRO BENZYL ETHER N ACETYL DI IODO
 TYROSINE POPULATION GROWTH

L19 ANSWER 3 OF 4 MEDLINE DUPLICATE 2
 ACCESSION NUMBER: 77025961 MEDLINE
 DOCUMENT NUMBER: 77025961 PubMed ID: 1086231
 TITLE: p-Azobenzene arsonate-L-tyrosine-mediated helper function in immune responses of guinea pigs and rats.
 AUTHOR: Becker M J; Ray A; Andersson L C; MAKELA O
 SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, (1975 Apr) 5 (4) 262-6.
 Journal code: EN5; 1273201. ISSN: 0014-2980.
 PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
 Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197701
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 19900313
Entered Medline: 19770103

- AB A small bifunctional antigen (4-hydroxy-5-iodo-3-nitrophenyl)
acetyl-epsilon-aminocaproyl-L-tyrosine
-azobenzene-p-arsonate [NIP-cap-TYR(ABA)] was found to induce fair humoral antibody formation against NIP-cap but very little anti-ABA-TYR. This was observed in rats and guinea pigs. Prior immunization with ABA-TYR, either as such or coupled to dodecanoylated bovine serum albumin (lipid-BSA), primed rats for an enhanced anti-NIP response to NIP-cap-TYR(ABA). An attempt to encourage rats to produce anti-ABA-TYR in response to the bifunctional antigen by priming them with NIP-cap-lipid-BSA failed. Priming with ABA-TYR was dose-dependent. An injection of 1.5-15 nanomoles per rat primed for an increased production of anti-NIP while 150 nanomoles did not. Adult thymectomized x-irradiated rats had a poor anti-NIP response to the bifunctional antigen if they were reconstituted with T-enriched lymphoid cells from control mice, but a good response if reconstituted with similar cells from ABA-TYR-primed syngeneic rats.
- AB A small bifunctional antigen (4-hydroxy-5-iodo-3-nitrophenyl)
acetyl-epsilon-aminocaproyl-L-tyrosine
-azobenzene-p-arsonate [NIP-cap-TYR(ABA)] was found to induce fair humoral antibody formation against NIP-cap but very little anti-ABA-TYR. This was observed in rats. . .

L19 ANSWER 4 OF 4 MEDLINE
ACCESSION NUMBER: 65130174 MEDLINE
DOCUMENT NUMBER: 65130174
TITLE: KINETICS OF IODINATION. II. GENERAL BASE CATALYSIS IN THE
IODINATION OF N-ACETYL-L-TYROSINE AND N-
ACETYL-3-iodo-L-TYROSINE.
AUTHOR: MAYBERRY W E; BERTOLI D A
SOURCE: JOURNAL OF ORGANIC CHEMISTRY, (1965 JUN) 30 2029-34.
Journal code: JIR. ISSN: 0022-3263.
PUB. COUNTRY: United States
Journal
LANGUAGE: English
FILE SEGMENT: OLDMEDLINE
ENTRY MONTH: 196511
ENTRY DATE: Entered STN: 19990716
Last Updated on STN: 19990716
TI KINETICS OF IODINATION. II. GENERAL BASE CATALYSIS IN THE IODINATION OF
N-ACETYL-L-TYROSINE AND N-**ACETYL-3-iodo-L-**
TYROSINE.

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